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Telomere length is repeatable, shortens with age and reproductive success, and predicts remaining lifespan in a long-lived seabird

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Abstract

Telomeres are protective caps at the end of chromosomes, and their length is positively correlated with individual health and lifespan across taxa. Longitudinal studies have provided mixed results regarding the within-individual repeatability of telomere length. While some studies suggest telomere length to be highly dynamic and sensitive to resource-demanding or stressful conditions, others suggest that between-individual differences are mostly present from birth and relatively little affected by the later environment. This dichotomy could arise from differences between species, but also from methodological issues. In our study, we used the highly reliable Terminal Restriction Fragment analysis method to measure telomeres over a 10-year period in adults of a long-lived seabird, the common tern (*Sterna hirundo*). Telomeres shortened with age within individuals. The individual repeatability of age-dependent telomere length was high (>0.53), and independent of the measurement interval (i.e., one vs. six years). A small (R^2 = .01), but significant part of the between-individual variation in telomere length was, however, explained by the number of fledglings produced in the previous year, while reproduction in years prior to the previous year had no effect. We confirmed that age-dependent telomere length predicted an individual's remaining lifespan. Overall, our study suggests that the majority of between-individual variation in adult telomere length is consistent across adult life, and that a smaller part of the variation can be explained by dynamic factors, such as reproduction.

KEYWORDS

ageing, life-history, mortality, parental investment, senescence, survival, terminal restriction fragment

1 | INTRODUCTION

Telomeres are highly conserved specialized nucleoprotein structures at the ends of eukaryotic chromosomes (Meyne, Ratliff, & Moyzis, 1989). They serve to maintain chromosome integrity (Blackburn, 1991), but shorten with cell replication and oxidative stress (Olovnikov, 1973; Stewart et al., 2003; von Zglinicki, 2002). Individuals with longer telomeres for their age are generally found to have better survival prospects, both in humans (reviewed by Boonekamp, Simons, Hemerik, & Verhulst, 2013) and other vertebrates, mainly mammals and birds (reviewed by Wilbourn et al., 2018). Explaining between- and within-individual variation in

telomere length therefore has become an important topic in the study of life histories.

Evidence is accumulating that telomere shortening with age can be increased due to various stressors or resource-demanding activities (e.g., Asghar et al., 2015; Haussmann, Longenecker, Marchetto, Juliano, & Bowden, 2012; Reichert et al., 2014; Salmon, Nilsson, Nord, Bensch, & Isaksson, 2016; Sudyka et al., 2019; Vedder, Verhulst, Zuidersma, & Bouwhuis, 2018; Wilbourn et al., 2017; reviewed in Angelier, Costantini, Blevin, & Chastel, 2018). This has led to the view that telomere length is a highly dynamic trait sensitive to environmental conditions, thereby acting as a biomarker for an individual's accumulated stress or somatic state (Monaghan, 2010; Monaghan & Haussmann, 2006). Studies showing that an individual's telomere length is difficult to predict from previous measurements – and is thus variably dynamic – are suggested to corroborate this view (Fairlie et al., 2016; van Lieshout et al., 2019; Martin-Ruiz, Gussekloo, van Heemst, von Zglinicki, & Westendorp, 2005; Spurgin et al., 2018; Svenson et al., 2011).

Other studies, however, have come to different conclusions. Some studies did not detect telomere shortening with age (e.g., Hoelzl, Cornils, Smith, Moodley, & Ruf, 2016; Hoelzl, Smith, et al., 2016). Moreover, in humans, a very high correlation ($r > .9$) between individual telomere length measurements with a more than 10-year interval suggests that human telomere length is a repeatable individual characteristic, although 10 years is a relatively small part of the average human lifespan (Benetos et al., 2013; Verhulst et al., 2016). Similarly, a study on free-living birds (jackdaws, *Corvus monedula*) showed that telomere lengths measured in adults are highly correlated with previous measures of the same individuals as chicks (Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014). In another free-living bird species, the common tern, (*Sterna hirundo*), there was little variation in telomere shortening between chicks (Vedder, Verhulst, Bauch, & Bouwhuis, 2017) and a high correlation between telomere lengths of adults measured at a one-year interval (Bauch, Becker, & Verhulst, 2014). Whether these results are due to a high repeatability of individual environmental conditions in these species, or to an insensitivity of telomere dynamics to environmental variation is currently unknown.

While the dichotomy in results regarding the strength of within-individual correlations in telomere length over time may be explained by species differences in the consistency of, or sensitivity to, environmental conditions, a methodological explanation cannot be excluded. Two popular methods used to measure telomere length are quantitative PCR (qPCR) and terminal restriction fragment (TRF) analysis. Whereas all of the above mentioned studies that found a high within-individual correlation in telomere length used the TRF method (Bauch et al., 2014; Benetos et al., 2013; Boonekamp et al., 2014; Vedder, Verhulst, et al., 2017), all but one of the studies that used the qPCR method reported low within-individual correlations (Fairlie et al., 2016; van Lieshout et al., 2019; Martin-Ruiz et al., 2005; Seeker et al., 2018; Spurgin et al., 2018; Svenson et al., 2011).

The qPCR method has the advantage of being relatively time- and cost-effective and is therefore often used to measure telomere length from large archived collections of stored DNA samples. However, the reliability of qPCR telomere measurement depends on the storage method (Eastwood, Mulder, Verhulst, & Peters, 2018; Nussey et al., 2014; Reichert et al., 2017), which may not have been chosen to ideally suit qPCR at the time of collection. Moreover, the qPCR method does not measure telomere length directly, but measures the ratio of telomeric DNA to a control gene sequence (Cawthon, 2002). Small measurement error in both of these quantities can magnify the error in the resulting ratio considerably, causing measurement error alone to produce a low correlation between successive measurements of individual telomere length (Nettle, Seeker, Nussey, Froy, & Bateson, 2019). The qPCR method also does not only measure the length of the telomere sequence at the end of chromosomes, but includes interstitial telomeric DNA, which can vary between individuals and can make up a considerable part (15%–40%) of the total telomeric DNA (Foote, Vleck, & Vleck, 2013).

The TRF method has the disadvantage of requiring high quality DNA, therefore needing more specific sampling and storage and making it less suitable for most existing DNA archives. It also requires a relatively large amount of DNA and can therefore be impractical to use in small animals. Moreover, it needs more specialized equipment, and is less time- and cost-effective. On the other hand, the TRF method has the advantage of measuring telomere length directly, yielding results expressed in base pairs that can numerically be compared between studies, and providing very reliable measurements (i.e., it is the gold standard; Nussey et al., 2014). However, to our knowledge, no study on free-living animals has used it to investigate telomere dynamics across a period that covers the major part of the lifespan of the species.

In this study, we used the TRF method to measure telomeres from adult common terns sampled in five separate years across a 10-year period. Analysis of the first two years of this period previously revealed telomere length to (a) shorten with age, (b) be highly correlated within individuals between the two years (2007 and 2008), and (c) be negatively correlated with reproductive success (Bauch, Becker, & Verhulst, 2013; Bauch, Riechert, Verhulst, & Becker, 2016). Adult telomere length was also suggested to predict an individual's remaining lifespan after sampling, but survival of sampled individuals was only followed for four years, and the overall effect did not reach statistical significance (Bauch et al., 2014). By extending the data set with three more years of sampling, and spanning a much larger time period, we are now in a position to: (a) accurately estimate within-individual rates of telomere shortening with age, (b) calculate intraindividual repeatability of telomere length over a long time interval, and compare within-individual correlations of telomere length over a one- and a six-year interval, (c) identify the reproductive stage that is associated with the greatest annual loss of telomeres, (d) assess whether the relative effect size of reproduction on telomere length diminishes, or accumulates, over time, and (e) confirm

that telomere length, corrected for age, predicts an individual's remaining lifespan.

2 | MATERIALS AND METHODS

2.1 | Species and study population

Common terns are socially monogamous migratory seabirds. The data for this study were collected as part of a long-term study of common terns in a monospecific breeding colony located at the "Banter See" in Wilhelmshaven, on the German North Sea coast (53°36′N, 08°06′E). The colony site consists of six concrete islands (10.7 × 4.6 m each) surrounded by 60 cm high walls that protect against flooding and prevent chicks from leaving the islands before fledging. Since 1992, all nests are checked three times a week to record clutch size, brood size and fledging success. Newly hatched chicks are ringed at their first encounter, between 0 and 2 days old, and receive a subcutaneously implanted transponder (model ID 100; TROVAN, Germany) shortly before fledging (Becker & Wendeln, 1997).

In common terns, both parents incubate, and marked parents are linked to their breeding attempts by placing an antenna (model EUR-3110; Euro I.D., Germany), which reads transponder codes every 5–10 s at a distance of ≤11 cm, around all clutches for at least 24 hr. The sex of locally-hatched breeders is molecularly determined from a feather sample, collected shortly before fledging, since 1998 (Becker & Wink, 2003) and was determined by behavioural observation before that. Common terns can rear one to three chicks per successful breeding attempt, and typically only have a second breeding attempt within a season if the first one fails (Becker & Ludwigs, 2004).

The walls around the islands support 44 resting platforms equipped with antennae that automatically record the presence of transponder-marked individuals. This allows the identification of subadults and breeding as well as nonbreeding adults, and a very accurate assessment of the annual adult survival rate (ca. 0.90) and individual lifespan (which, for adults, is on average 10 years) (Ezard, Becker, & Coulson, 2006; Szostek & Becker, 2012). For this study, we assumed a breeder had died if it was not registered for at least two consecutive years, until the breeding season of 2018. The reliability of this assumption is high, since 97% of breeders do not skip registration for more than two consecutive years (Zhang, Vedder, Becker, & Bouwhuis, 2015).

2.2 | Blood sampling

Blood samples were collected in 2007, 2008, 2013, 2014 and 2016 using a larval instar of the blood-sucking bug *Dipetalogaster maximus*. To collect a sample from a specific bird, a bug was placed in a hollow artificial egg that was temporarily placed in the nest of that bird during incubation (for details see Arnold et al., 2008). Visual observation combined with the antenna system was used to confirm the continuous presence of the bird. After 15–20 min, the artificial egg was retrieved and the blood extracted and stored in 2% EDTA buffer. Blood samples were kept at 4°C until transferred into a 40% glycerol buffer within three weeks after sampling and then snap-frozen at −80°C until analysis. It was previously confirmed that blood-sampling with bugs does not affect the telomere measurements, as compared to blood sampling with a needle (Bauch et al., 2013).

2.3 | Telomere measurements

From each blood sample, average erythrocyte telomere length was measured by in-gel terminal restriction fragment (TRF) analysis without DNA denaturation (following Bauch et al., 2013; Vedder et al., 2018), which excludes interstitial telomeric sequences. The glycerol buffer was removed and the blood cells were washed with 2% EDTA. DNA was isolated from erythrocytes (7 µl cells) using a CHEF Genomic DNA plug kit (Bio-Rad) and subsequently digested simultaneously with *Hind III* (60U), *Msp I* (60U) and *Hinf I* (30U) in NEB2 buffer (New England Biolabs, Inc.) at 37°C for c. 18 hr. One third of the DNA from every sample was added in a 0.8% nondenaturing agarose gel (Pulsed Field Certified Agarose, Bio-Rad) and separated by pulsed field electrophoresis for 22 hr at 14°C (3 V/cm, initial switch time 0.5 s, final switch time 7.0 s). Two differently-sized ^{32}P-labelled ladders were added for size calibration on every gel: (a) a 1 kb DNA ladder (range 1–10 kb; New England Biolabs, Inc.) (once per gel) and (b) DNA Molecular Weight Marker XV (range 2–48 kb; Roche Diagnostics) (twice per gel). In addition, we added a standard sample (DNA extracted from a single blood sample of a single chicken; once per gel) as a control. Gels were dried with a gel dryer (model 538, Bio-Rad) and hybridised overnight at 37°C with a ^{32}P-labelled oligonucleotide $(5'\text{-}C_3TA_2\text{-}3')_4$ that binds to the telomeric single-strand overhang. Subsequent washing of the gel with 0.25 × SSC buffer at 37°C removed unbound oligonucleotides. The gel was then exposed to a phosphor screen for 4 hr (MS, PerkinElmer, Inc.) to detect the radioactive signal, which was visualised by a phosphor imager (Cyclone Storage Phosphor System, PerkinElmer, Inc.). Telomere length distributions were quantified using ImageJ (version 1.38x, open source) and the average telomere length (in base pairs) was calculated for every sample following the method described in Bauch et al. (2013). The lower limit of the telomere length distribution was lane-specifically set at the point with the lowest signal. At the upper end of the distribution, we set a fixed limit at 30 kb because the background noise in the region of the longest telomeres was more variable. This selected range was previously shown to represent the telomere distribution with the highest intraindividual repeatability for average telomere length in common terns (Bauch et al., 2013). The background value was subtracted lane-specifically from the optical density measurements.

The coefficient of variation of the standard sample run on all gels (n = 58) was 0.06. We previously found an intrablood sample repeatability (with multiple DNA extractions per sample) of average telomere length of 0.86 when measuring telomere length of common tern chicks using the same method (Vedder et al., 2018).

In total, we collected 619 telomere measurements from 387 individuals, aged two to 24 years (see Appendix S1: Figure S1 for a histogram of the age distribution). Among them, 220 individuals had one measurement of telomere length, 122 individuals had two measurements in different years, 27 had three, 16 had four, and two individuals had five. For 81% of the individuals with repeated measures (n = 167), repeats were not all analysed on the same gel, facilitating reliable separation of individual differences and "gel effects" (see Section 2.4).

2.4 | Statistical analysis

2.4.1 | Shortening with age

To test whether telomere length shortened with age, we ran a linear mixed model (LMM) with telomere length as the dependent variable and an individual's actual age partitioned into average age and delta age as explanatory variables (following van de Pol & Wright, 2009). An individual's average age was calculated as the average of all ages at which we measured an individual's telomere length, while delta age was calculated as the difference between an individual's actual age at measurement and its average age (i.e., delta age = age − average age). In this way, average age represents the between-individual age effect, which corrects for potential selective disappearance of birds with short or long telomeres, while delta age represents the within-individual age effect on telomere length (van de Pol & Wright, 2009). In order to test whether the between- and within-individual age effects were significantly different, which would indicate selective disappearance, we ran a similar model including both actual age and average age as explanatory variables. In this model, the actual age term represents the within-individual effect, while the average age term represents the difference between the between- and within-individual effects (van de Pol & Wright, 2009). Both models included the sex of the bird and "lab" as categorical fixed effects with two levels. The "lab" term was added to capture the fact that the measurements of samples collected in 2016 were done with different equipment and by a different person, which may have led to slight modifications in outcome. Gel and bird identity were added as random effects. In addition to additive effects, we considered first order interactions involving average age, delta age (or age) and sex, and quadratic effects of average age and delta age (or age). Although we may not expect sex-specific effect because both sexes incubate, brood and provision chicks, there are small differences in parental care between the sexes. When the chicks are still small (<1 week old) males do more provisioning, while females spend more time at brooding (Becker & Ludwigs, 2004).

2.4.2 | Intraindividual repeatability

The intraindividual repeatability of telomere length was calculated using the R package RPTR (Stoffel, Nakagawa, & Schielzeth, 2017) with the 95% confidence interval (95% CI) being obtained from 1,000 bootstrap iterations. The intraindividual repeatability is computed by dividing the between-individual variance in telomere length by the total variance, after accounting for fixed effects. This model was fitted with a normal error distribution and included fixed effects of average age, delta age, sex and "lab", and random effects of gel and bird identity. This way, we simultaneously calculated the intragel repeatability of telomere length (by dividing the between-gel variation by the total variation) and in the Results we present the intraindividual repeatability obtained when the between-gel variation is or is not included in the total variation.

To test whether the relationship between an individual's telomere lengths at two ages diminished with a longer age interval between measurements, we tested the strength of the correlation between individual telomere length measured at one year (2007–2008 and 2013–2014, n = 126 intervals from 114 individuals) and at six year (2007–2013 and 2008–2014, n = 53 intervals from 41 individuals, of which 39 [95%] were also included in the one-year interval analysis) intervals. These intervals were chosen because we particularly sampled many individuals repeatedly in 2007–2008 and 2013–2014. In two different LMMs, the telomere length of the same individual measured one or six years earlier, respectively, was added as an explanatory variable, while gel identity and bird identity were added as random effects. We did not include "lab" because no samples of 2016 were incorporated in these analyses. The standardized effect size (±95% CI) of the earlier telomere length was calculated as the semi-partial R square (R^2), following Nakagawa and Schielzeth (2013), implemented in the R package 'R2GLMM' (Jaeger, Edwards, Das, & Sen, 2017).

2.4.3 | Effect of reproduction

To investigate whether reproductive success in one year affects telomere length in the following year, we added the total number of fledglings produced in the previous year (regardless of whether these fledglings originated from the first clutch or from a replacement clutch) as an explanatory variable in model described above. This new LMM had a reduced sample size (n = 545 measurements, from 333 individuals), because birds that were sampled at their first reproductive attempt (n = 65) or birds that skipped reproduction in the year before sampling (n = 9) were not included. As in the previous model, sex of the bird and "lab" were included as fixed effects, gel and bird identity as random effects. In addition to additive effects, we considered first-order interactions between reproductive success and age (delta age and average age), as well as between reproductive success and sex.

To investigate from which breeding stage the observed effect of reproduction on telomere length originated (see (see Section 3), we ran five models that tested for effects of five underlying stages: the size of the first, or only, clutch in the previous year (clutch size), the number of hatchlings of that clutch (brood size d0), the brood size at 10 and 18 days after hatching of that clutch (brood size d10 and

d18, respectively), and the number of fledglings of that clutch (see Bauch et al., 2013 for a similar approach). The semi-partial R square (R^2) of each breeding stage variable was calculated as above.

To assess whether the effect of annual reproduction on telomere length diminishes, or accumulates, over time, we also tested whether annual reproductive success (total number of fledglings) in years prior to the previous year, or in the current year, explained variation in telomere length. Details of the methods, sample sizes and results of these analyses are presented in the Supporting Information (Appendix S2: Table S1).

To specifically test whether increased reproductive success leads to increased shortening of telomeres within individuals, we ran a LMM with telomere length as the dependent variable and fixed effects of sex, the total number of fledglings in the previous year, and telomere length in the previous year, for the individuals that were measured in at least two consecutive years (n = 126 telomere measurements from 114 individuals). Doing so allowed the telomere length in the previous year to correct for all previous telomere-length associated variation between individuals, causing the reproductive success term to reflect an effect on the change in telomere length from one year to the next. This model included gel and bird identity as random effects.

2.4.4 | Remaining lifespan and survival

Using the telomere measurements of individuals for which we have complete lifespans (n = 243 measurements from 161 individuals), we analysed whether an individual's telomere length predicts its remaining lifespan. To this end, we ran a Generalized Linear Mixed Model (GLMM) with a log link function and a Poisson error distribution. Remaining lifespan was the dependent variable and defined as the number of years between an individual's age of telomere measurement and the age at which it was last recorded in the colony. Telomere length (standardized), age (standardized) and sex were included as fixed effects, bird identity as a random effect. Additive effects of all fixed effects, as well as all first order interactions, were considered. In this model, age was not partitioned into average age and delta age, because it would not make sense to test for effects of selective disappearance on remaining lifespan (which itself reflects selective disappearance).

To be able to make use of the complete data set of 619 telomere measurements of 387 individuals, we also tested whether the mortality hazard of individuals depends on their telomere length with a mixed-effects Cox right-censored regression model (Nenko, Hayward, Simons, & Lummaa, 2018; Ripatti & Palmgren, 2000; Therneau, Grambsch, & Pankratz, 2003). All the details regarding this analysis and its associated results are given in the Supporting Information (Appendix S3: Table S2).

All analyses were performed in R 3.4.2 (R Core Team, 2014) using the functions lmer for the LMMs and glmer for the GLMM, implemented in the package LME4 (Bates, Maechler, Bolker, & Walker, 2015) using restricted maximum likelihood estimates of the parameters. The GLMM was tested for over-dispersion using the function dispersion_glmer implemented in the package BLMECO (Korner-Nievergelt et al., 2015); the ratio was below the recommended threshold of 1.40 (1.06). We present models without interaction terms to allow a straightforward interpretation of the effect of single terms. Models with interaction terms and quadratic age effects (which were always nonsignificant) are presented in the Supporting Information (Appendix S4: Tables S3, S4 and S5). Parameter estimates are given as the mean ± 1 standard error (*SE*).

3 | RESULTS

3.1 | Shortening with age

There was a trend towards telomere length being shorter in females than males (β = −108.49 ± 60.48 bp, t = −1.79, p = .07). Moreover, telomere length negatively correlated with age, both within (β = −60.57 ± 12.44 bp/year, t = −4.87, p < .001) and between individuals (β = −29.82 ± 8.73 bp/year, t = −3.42, p < .001) (Figure 1, Table 1). These age effects did not differ between the sexes and were linear, not quadratic (Table S3). There was a trend towards the between-individual average age effect being less negative than the within-individual delta age effect (β = 30.74 ± 16.25, t = 1.89, p = .06, Table 1), suggesting individuals with short telomeres selectively disappear from the sampled population (see below). The "lab" effect was significant (β = 876.44 ± 148.06, t = 5.92, p < .001, Table 1), confirming the relevance of correcting for it.

3.2 | Intraindividual repeatability

The repeatability of telomere length within individuals was 0.53 (±0.04, 95% CI = 0.45–0.61, p < .001). In addition, samples on the

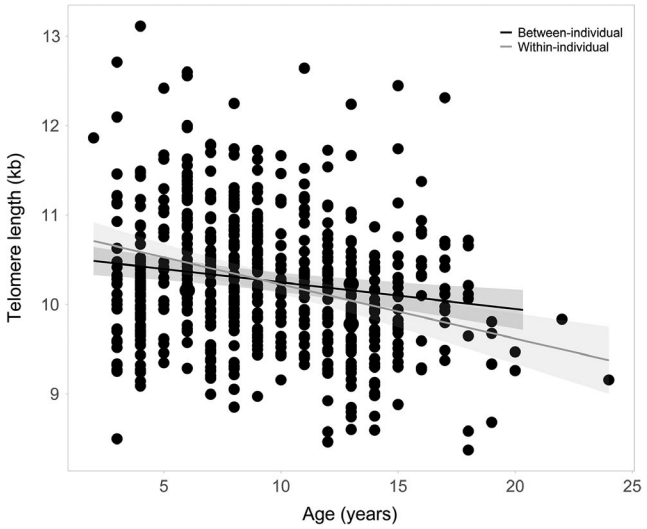

FIGURE 1 Telomere length plotted against age. The dots represent the individual telomere length measurements. The solid lines represent the model predictions with their standard errors (grey areas), with the black line representing the between-individual age effect, and the grey line the within-individual age effect

TABLE 1 Effect of age on telomere length. Parameter estimates were obtained from full models, without interaction terms and quadratic effects (see Table S3 for the model with interaction terms and quadratic effects)

Dependent variable	Telomere length (in bp, *n* = 619 measurements from 387 individuals)					
Parameter	**Estimate ± *SE***	***t* value**	***p*-value**	**Estimate ± *SE***	***t* value**	***p*-value**
Intercept	**10,474.95 ± 103.02**	**101.68**	**<.001**	**10,474.95 ± 103.02**	**101.68**	**<.001**
Age	–	–	–	**−60.57 ± 12.44**	**−4.87**	**<.001**
Average age	**−29.82 ± 8.73**	**−3.42**	**<.01**	30.74 ± 16.25	1.89	.059
Delta age	**−60.57 ± 12.44**	**−4.87**	**<.001**	–	–	–
Sex (female)	−108.49 ± 60.48	−1.79	.074	−108.49 ± 60.48	−1.79	.074
Lab	**876.44 ± 148.06**	**5.92**	**<.001**	**876.44 ± 148.06**	**5.92**	**<.001**
Random bird ID (variance ± *SD*)	253,766.00 ± 503.80			253,766.00 ± 503.80		
Random gel ID (variance ± *SD*)	108,259.00 ± 329.00			108,259.00 ± 329.00		

Note: Significant effects ($p < .05$) are in bold and '–' means that a parameter was not fitted to the model.
Abbreviations: *SD*, standard deviation; *SE*, standard error.

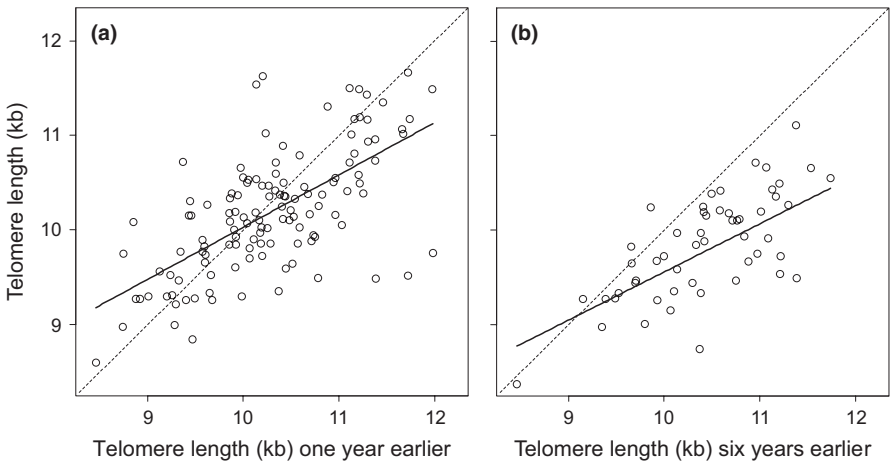

FIGURE 2 The correlation between two measures of an individual's telomere length (a) with a one-year interval and (b) with a six-year interval. The dots represent the individual telomere length measurements, the black lines the model predictions, and the dashed lines the $y = x$ lines that represent no change

same gel were also significantly repeatable (0.22 ± 0.04, CI 95%: 0.14–0.31, $p < .001$), indicating that there was variation between gels that was not explained by differences between individuals measured on these gels. Hence, if we assume that significant between-gel variation reflects measurement error instead of biological variation, the actual repeatability of telomere length may be even higher. Subtracting the 22% of variation explained by gel identity from the total variation would lead to the intraindividual repeatability of telomere length being 0.68 (i.e., 0.53/[1–0.22]).

The effect of an individual's telomere length on that in the next year was highly significant (Figure 2a, $\beta = 0.53 \pm 0.06$, $t = 9.54$, $p < .001$). This effect was also highly significant when the interval was not one, but six years (Figure 2b, $\beta = 0.51 \pm 0.07$, $t = 7.31$, $p < .001$). In fact, standardized effect sizes did not differ between a one-year ($R^2 = .43$, CI 95%: 0.31–0.54) and a six-year interval ($R^2 = .45$, CI 95%: 0.27–0.61).

3.3 | Effect of reproduction

An individual's telomeres were shorter if it had produced more fledglings in the previous year ($\beta = -112.14 \pm 31.97$ bp/fledgling, $t = -3.51$, $p < .001$, Figure 3a, Table 2). The association between reproductive success and telomere length did not differ between the sexes or vary with age (Table S4). The effect of the number of fledglings in the previous year was also significant when correcting for individual telomere length in the previous year, indicating an effect of reproductive success on individual telomere shortening ($\beta = -117.83 \pm 41.15$ bp/fledgling, $t = -2.86$, $p = .005$). The effect of reproductive success on telomere length originated after chicks hatched, because clutch size and brood size at hatching had no effect on telomere length, while brood size at days 10 and 18 and the number of fledglings from the first clutch had an increasingly strong effect (Figure 3, Table 2). Individual reproductive success in years prior to the previous year, and in the current year (after sampling), were not associated with telomere length (Table S1).

3.4 | Remaining lifespan and survival

Correcting for age, individuals with longer telomeres had a longer remaining lifespan after telomere measurement ($\beta = 0.16 \pm 0.07$, $Z = 2.28$, $p = .02$, Figure 4, Table 3). This confirms that the suggested

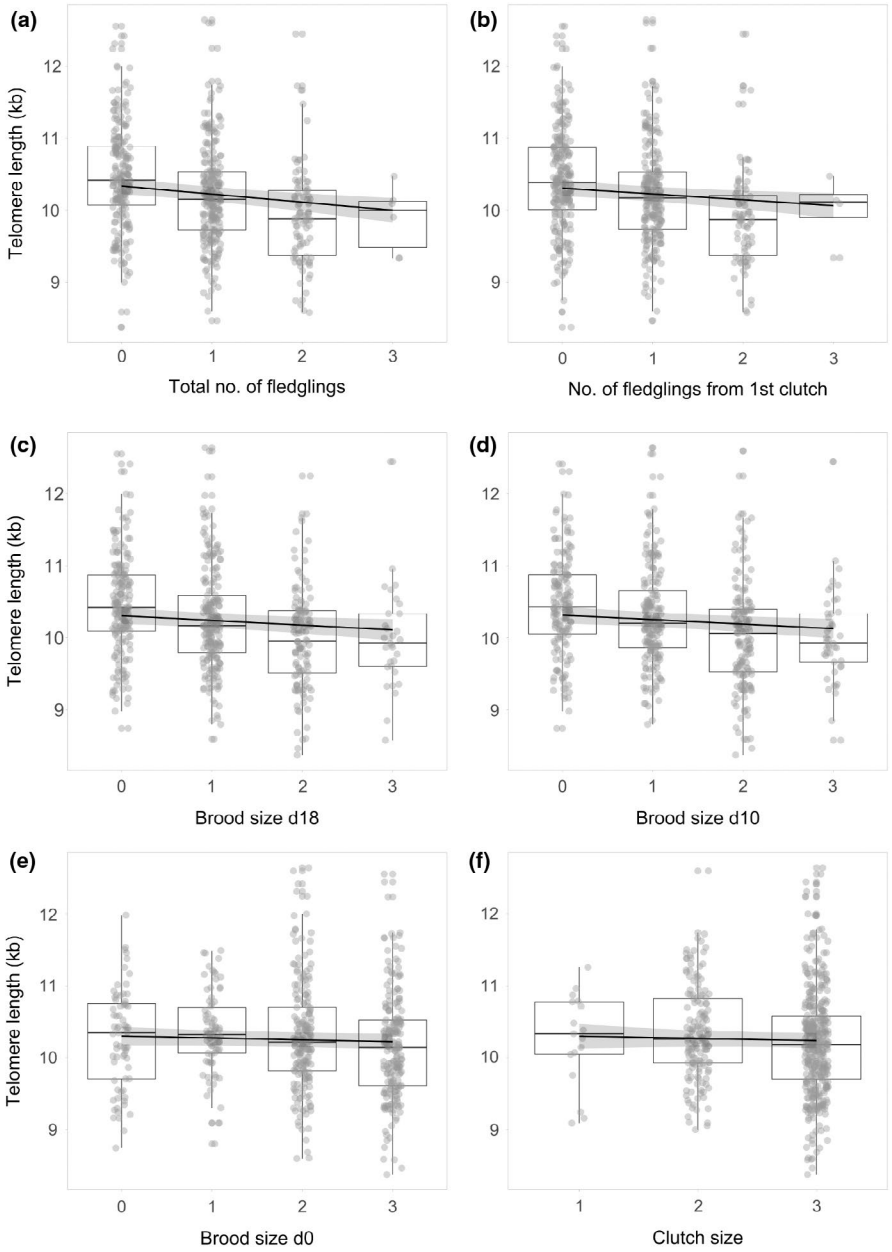

FIGURE 3 Boxplots plotting telomere length against the previous year's (a) total number of fledglings produced in the season, (b) number of fledglings of the first clutch, brood size at (c) d18, (d) d10, (e) d0 and (f) clutch size of the first clutch. The lower and upper hinges correspond to the first and third quartiles, the bar represents the median. The whiskers extend from the hinge to the largest/smallest values no further than/at most 1.5 × IQR. The dots represent the individual telomere length measurements. The solid lines represent the model predictions with standard error (grey areas)

selective disappearance of individuals with short telomeres from the population (see above) is the result of increased mortality of individuals with short telomeres. The association between telomere length and residual lifespan did not differ between the sexes or vary with age (Table S5). In accordance with the effect on remaining lifespan we found a negative effect of telomere length on an individual's mortality hazard when we tested a mixed-effects right-censored Cox model on the full data set (hazard ratio <1, $n = 619$, $p = .03$, Table S2).

4 | DISCUSSION

Using repeated telomere measurements from common terns ranging in age between two and 24 years, we found telomere length to decline with age. This decline could be attributed to a within-individual shortening of, on average, 61 bp per year, because our longitudinal data allowed us to separate within-individual age effects from between-individual effects arising from selective disappearance. In our case, not correcting for selective disappearance would have led to an underestimation of individual telomere shortening with age, since individuals with short telomeres were under-represented among the older age classes (see below). Although there is uncertainty about whether telomere length in somatic cells can only decrease with age (Bateson & Nettle, 2017; Haussmann & Mauck, 2008; Hoelzl, Cornils, et al., 2016; Hoelzl, Smith, et al., 2016), our observation that practically all individuals with measurements across a six year interval showed a decrease in telomere length (Figure 2b) suggests that common terns do not prevent telomere loss in the long term.

We found that at least 53% of the variation in telomere length was due to consistent differences between individuals. This percentage may, however, be closer to 68% if we assume that any consistent between-gel variation does not represent biological variation.

TABLE 2 Effect of reproductive success on telomere length. Parameter estimates were obtained from full models, without interaction terms (see Table S4 for the model with interaction terms)

Dependent variable	Telomere length (in bp, *n* = 545 measurements from 333 individuals)							
Reproductive success variable	Total number of fledglings			Number of fledglings of the first clutch			Brood size d18 of the first clutch	
Parameter	Estimate ± *SE*	*t* value	*p*-value	Estimate ± *SE*	*t* value	*p*-value	Estimate ± *SE*	*t* value
Intercept	**10,535.41 ± 114.38**	**92.11**	**<.001**	**10,523.20 ± 114.50**	**91.90**	**<.001**	**10,519.93 ± 114.50**	**91.87**
Average age	**−25.43 ± 9.95**	**−2.56**	**.011**	**−27.48 ± 9.98**	**−2.75**	**.006**	**−26.98 ± 10.04**	**−2.69**
Delta age	**−60.69 ± 12.58**	**−4.83**	**<.001**	**−63.15 ± 12.68**	**−4.98**	**<.001**	**−64.91 ± 12.57**	**−5.17**
Reproductive success	**−112.14 ± 31.97**	**−3.51**	**<.001**	**−81.16 ± 32.67**	**−2.48**	**.013**	**−65.72 ± 27.54**	**−2.39**
Sex (female)	−115.16 ± 65.48	−1.76	.080	−112.44 ± 65.50	−1.72	.087	−110.48 ± 65.51	−1.69
Lab	**736.53 ± 137.55**	**5.36**	**<.001**	**752.52 ± 138.54**	**5.43**	**<.001**	**759.46 ± 138.70**	**5.48**
Random bird ID (variance ± *SD*)	262,232.00 ± 512.10			260,225.00 ± 510.10			260,199.00 ± 510.10	
Random gel identity (variance ± *SD*)	80,346.00 ± 283.50			81,824.00 ± 286.00			82,456.00 ± 287.20	
R^2 for the reproductive success (CI 95%)	.013 (0.001–0.038)			.007 (0.000–0.027)			.006 (0.000–0.026)	

Significant effects ($p < .05$) are in bold.

Abbreviations: *SD*, standard deviation; *SE*, standard error.

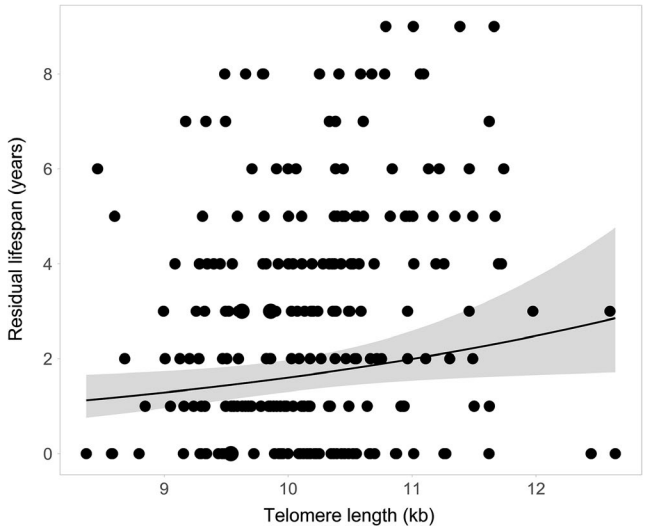

FIGURE 4 Remaining lifespan plotted against telomere length at sampling. The dots represent the individual telomere length measurements. The solid line represents the model prediction with standard error (grey area) corrected for age effects

TABLE 3 Effects of telomere length, age and sex on residual lifespan. Parameter estimates were obtained from full models without interaction terms (see Table S5 for the model with interaction terms)

Dependent variable	Residual lifespan (*n* = 243 measurements from 161 individuals)		
Parameter	Estimate ± *SE*	*Z* value	*p*-value
Intercept	**0.70 ± 0.13**	**5.32**	**<0.001**
Standardized telomere length	**0.16 ± 0.07**	**2.28**	**0.023**
Standardized age	**−0.55 ± 0.11**	**−5.15**	**<0.001**
Sex (female)	0.10 ± 0.17	0.57	0.569
Random bird ID (variance ± *SD*)	0.80 ± 0.89		

Note: Significant effects ($p < .05$) are in bold.

Abbreviations: *SD*, standard deviation; *SE*, standard error.

The between-gel variation, observed after statistically accounting for these gels containing different individuals, in combination with the observed "lab" effect, suggests that, even with the TRF method, measurement error may be unavoidable and that we cannot exclude some degree of measurement error within gels, for which we cannot correct statistically. As such, the intraindividual repeatability we report should be treated as a lower limit to the true intraindividual repeatability of telomere length. This suggests that individuals are rather consistent in how they rank compared to others in the population, with respect to their telomere length. We also showed that this within-individual consistency did not diminish over time, as the within-individual correlation of telomere length was very similar across a six year and a one year interval. This result resembles that of long-term studies in humans that found even higher within-individual correlations over a longer time period (but note that six years represents more than half of a common tern's average lifespan, while ~11 years does not for humans) (Benetos et al., 2013; Verhulst et al., 2016).

Our results corroborate other, shorter term, longitudinal studies using the TRF method on wild populations that found high within-individual consistency (Boonekamp et al., 2014; Vedder, Verhulst, et al., 2017). However, they contrast with those obtained in long-term studies that used qPCR to measure telomeres in the wild. In Soay sheep

	Brood size d10 of the first clutch			Brood size d0 of the first clutch			Clutch size of the first clutch		
p-value	Estimate ± *SE*	*t* value	*p*-value	Estimate ± *SE*	*t* value	*p*-value	Estimate ± *SE*	*t* value	*p*-value
<.001	**10,534.49 ± 115.00**	**91.61**	**<.001**	**10,533.98 ± 117.92**	**89.33**	**<.001**	**10,572.89 ± 150.76**	**70.13**	**<.001**
.008	**−27.52 ± 9.98**	**−2.76**	**.006**	**−30.14 ± 10.01**	**−3.01**	**<.01**	**−30.90 ± 10.03**	**−3.08**	**<.01**
<.001	**−64.51 ± 12.57**	**−5.13**	**<.001**	**−67.50 ± 12.56**	**−5.37**	**<.001**	**−66.97 ± 12.76**	**−5.25**	**<.001**
.017	**−62.78 ± 25.33**	**−2.48**	**.014**	−26.28 ± 23.38	−1.12	.262	−31.19 ± 43.61	−0.72	.475
.093	−111.12 ± 65.36	−1.70	.090	−112.69 ± 65.60	−1.72	.087	−112.82 ± 65.80	−1.71	.087
<.001	**757.55 ± 140.08**	**5.41**	**<.001**	**806.12 ± 141.14**	**5.71**	**<.001**	**796.24 ± 140.64**	**5.66**	**<.001**
	258,513.00 ± 508.40			259,988.00 ± 509.90			262,369.00 ± 512.20		
	84,794.00 ± 291.20			86,374.00 ± 293.90			86,370.00 ± 293.90		
	.006 (0.000–0.027)			.001 (0.000–0.015)			.001 (0.000–0.012)		

(*Ovis aries*; Fairlie et al., 2016), Seychelles warblers (*Acrocephalus sechellensis*; Spurgin et al., 2018) and European badgers (*Meles meles*; van Lieshout et al., 2019), the within-individual repeatabilities were 0.13, 0.07 and 0.03 respectively, indicating that the vast majority of variation in telomere length could not be attributed to consistent between-individual differences. While the latter pattern may suggest telomere length to be a dynamic trait that is highly susceptible to environmental stressors in these species, a lack of knowledge regarding the repeatability of an individual's environmental conditions or stress would make such an inference premature. In the common tern, we expect the within-individual repeatability of stress, or other costly activities, to be rather low, as life history data suggest comparatively little between-individual heterogeneity in quality (Vedder & Bouwhuis, 2018). As such, we believe that other explanations for the discrepancy in results among long-term studies on wild populations deserve further investigation. Studies on birds, for example, measure erythrocyte telomere length, while studies on mammals measure leucocyte telomere length, and it cannot be excluded that these tissues differ in their telomere dynamics (Beaulieu, Benoit, Abaga, Kappeler, & Charpentier, 2017). Moreover, little is known about the dynamics of interstitial telomeric DNA, which is included in qPCR measures of telomere length. A large difference in the amount of measurement error between measurement methods would also provide an explanation, but some qPCR studies report intrasample repeatabilities that are similar to those obtained in TRF studies (e.g., Eastwood et al., 2019; van Lieshout et al., 2019). More studies with repeatedly sampled individuals over a long time period will be required to resolve this issue.

We found a negative association between past reproduction and telomere length, a pattern that was also found in a variety of other species (Heidinger et al., 2012; Kotrschal, Ilmonen, & Penn, 2007; Reichert et al., 2014; Sudyka et al., 2014, 2019). However, most of these studies (but see Sudyka et al., 2019) experimentally manipulated reproductive effort (Kotrschal et al., 2007; Reichert et al., 2014; Sudyka et al., 2014), or were conducted in captivity (Heidinger et al., 2012) with relatively little variation in resource availability. In contrast, observational studies on wild populations have mostly reported positive associations between reproductive success and telomere length (Angelier, Weimerskirch, Barbraud, & Chastel, 2019; Le Vaillant et al., 2015; Parolini et al., 2017). Such differences between experimental effects and natural patterns can be explained by the large between-individual variation in resource acquisition observed in the wild (Vedder & Bouwhuis, 2018), because if variation in resource acquisition exceeds that in resource allocation, resource-based trade-offs are masked and positive associations are predicted (van Noordwijk & de Jong, 1986). Since between-individual variation in resource acquisition seems comparatively low in common terns (Vedder & Bouwhuis, 2018), this can explain why we were able to reveal the trade-off between reproductive success and telomere length under natural conditions.

We found that reproduction only affected the telomere length of parents if chicks survived to at least 10 days old. This is the stage when the energetic demand of chicks increases (Vedder, Zhang, & Bouwhuis, 2017), and before which many chicks die, especially in years with low food availability (Vedder, Zhang, Dänhardt, & Bouwhuis, 2019). As such, our results suggest that telomere shortening serves as a good proxy for the cost of reproduction (also see Bauch et al., 2013; Bauch et al., 2016), and that parents may largely escape this cost if their chicks die early (also see Vedder et al., 2019). Moreover, we found that only reproductive success in the previous year affected telomere length, while reproductive success in years prior to that had no effect (see Supporting Information for details).

This contrasts with studies that observed an effect of reproductive investment after more than a year (Reichert et al., 2014), a cumulative effect of reproductive events (Ryan et al., 2018) or an effect of lifetime reproductive success (Sudyka et al., 2019). Since reproductive success only explained a very small part of the variation in adult telomere length in common terns (R^2 = .01), other environmental factors may be more important in this species. These factors, as yet unknown, may have diluted any longer-term effect of reproduction on telomere length to the extent that it was no longer detectable.

In agreement with other studies (Wilbourn et al., 2018), we found that longer telomere length, corrected for age, predicted a longer remaining lifespan and a lower mortality hazard in adults. Combined with the high within-individual repeatability in telomere length, this raises the intriguing question of whether an individual's telomere length and lifespan are already partly determined early in life (Eastwood et al., 2019; Heidinger et al., 2012). In the same colony of common terns, we were previously unable to identify environmental factors that affected telomere length after hatching (Vedder, Verhulst, et al., 2017), but conditions during embryonic development (Vedder et al., 2018), as well as paternal age (Bouwhuis, Verhulst, Bauch, & Vedder, 2018; see also Bauch, Boonekamp, Korsten, Mulder, & Verhulst, 2019 for a longitudinal study in jackdaws *Corvus monedula*), affected telomere length in common tern chicks. If both telomere length and adult lifespan are particularly susceptible to such factors early in life (Bouwhuis, Vedder, & Becker, 2015), this can explain the high within-individual consistency in telomere length combined with its ability to predict adult lifespan. Alternatively, the variation in telomere length at hatching does not predict lifespan, but the variation that accrues over life via the effect of stressors and costly activities such as reproduction does. Indeed, some studies suggest that the rate of telomere shortening is more predictive of lifespan than the variation in telomere length at baseline measurement (Bize, Criscuolo, Metcalfe, Nasir, & Monaghan, 2009; Boonekamp et al., 2014; Epel et al., 2009; Salomons et al., 2009). A formal quantitative analysis that can partition variation in telomere length across genetic, parental and environmental effects, and estimate each component's association with lifespan, can resolve this question, but no data set may currently be sufficiently large and accurate to achieve this goal (Dugdale & Richardson, 2018).

Overall, our long-term longitudinal study revealed that telomeres shorten with age within individuals, and that the majority of the between-individual variation in adult telomere length is consistent across adult life. A smaller part of the between-individual variation should be explained by other factors, of which one is reproduction in the previous year. Costs of reproduction were, however, only reflected in telomere length if chicks survived through the first days of the chick stage, after which they become more energetically costly to raise. Adult telomere length, corrected for age, predicted remaining lifespan, and we suggest future studies focus on identifying the sources of variation in telomere length that are responsible for this pattern.

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AUTHOR CONTRIBUTIONS

O.V., S.B., C.Bi., P.H.B., and S.V. designed the study; S.B., C.Ba and O.V. collected blood samples; C.Ba. measured telomere lengths; C.Bi. analysed the data with the help of O.V. and S.B.; C.Bi. and O.V. wrote the paper with contributions from all other authors.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository – https://doi.org/10.5061/dryad.r24352h.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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